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Mini Review

Trends in Three-dimensional Biochips

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Abstract

In the past decade we have witnessed a rapid development in nano biotechnology that has yielded high-throughput and large-scale analysis within a miniaturized and parallel assay system. The biochip is a powerful emerging technology for biomedical, diagnostical, therapeutical, toxicological, and environmental applications. There are diverse sensing mediators used for biochips, including DNA, RNA, and protein, and moreover, even living cells are being studied. Recently, a large variety of biochip materials have been developed. Here, we will give an overview of the trends in three-dimensional biochip materials, particularly focusing on gel materials in biochip applications. We will review some unique methods and remaining challenges.

Keywords: Three-dimensional biochip, Sol-gel, Microarray, Aptamer, SELEX

The biochip is a powerful emerging technology for a large-scale and high-throughput collection and analysis of information. A biochip contains a series of miniaturized test sites (microarrays) arranged on a solid platform that allows thousands of individual experiments to be performed in-parallel at the same time. The development and application of biochips has become a hot issue in many research fields such as genomics, proteomics, pharmacology, material science, and computational bioscience, which require biotechnology by means of their research tools. The miniaturized sizes and high sensitivity of microarrays, which include a DNA microarray[1], protein microarray[2], chemical compound microarray[3], antibody microarray[4], living cell microarray[5], and tissue microarray[6], will revolutionize researches in medicine, diagnosis, therapeutics, toxicology, environmental monitoring, forensics, and so on.

Since the last decade, three-dimensional gel materials have been widely investigated for encapsulation of biological species such as enzymes, antibodies, and other proteins with functional sites[7]. Three-dimensional gel materials, including sol-gel or hydrogel, are regarded as a suitable encapsulation matrix for biomolecules with several advantages[7]. In a three-dimensional structure, small molecules tend to diffuse into the matrix, while large ones remain trapped there without any covalent bonding or modification of biomolecules[7]. Based on the versatility of gel-derived biocomposites, the gel materials provide a series of significant advantages for the development of advanced analytical instruments, such as biochips.

In this review, we will give an overview of various kinds of three-dimensional microarrays, their applications, and synthetic methods, as well as provide a discussion of some remaining challenges.

Use of Three-dimensional Chips in DNA Microarrays

DNA chips refer to microscopic spots of DNA oligonucleotides that are bound to a solid surface by a covalent attachment to a chemical matrix and the hybridization of complementary sequences to the sample.

The preparation of DNA microarrays involves the synthesis of an oligonucleotide sequence that can either be presynthesized and spotted onto a surface or directly synthesized onto it (in-situ synthesis)[1]. In both cases, immobilization of these oligonucleotides plays an important role in the synthesis. The CombiMatrix company developed a biocompatible Porous Reaction Layer (PRL)[8] (Figure 1) for immobilization of oligonucleotides. The chemical compound coated on a microarray platform acts as the supporting material for the oligonucleotides, which will subsequently be synthesized at the desired spots using in-situ synthesis. Compared to conventional spotting or in-situ methods, this in-situ method provides separate reaction tubes to synthesize different products in individual tubes.

In this way, a parallel analysis of data with high specificity drastically reduces the cost and time to examine diverse combinations of sequences. Due to the high accuracy resulting from the hybridization specificity of oligonucleotides in a polymer reaction layer, this three-dimensional DNA chip can be applied in many areas, i.e, in. identification for diagnostic

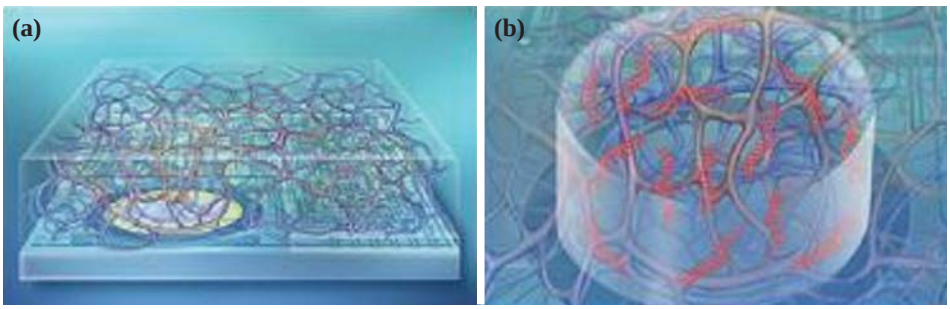

Figure 1. Structure of CombiMatrix DNA chip: (a) Porous Reaction Layer (PRL) that supports the attachment of synthesized DNA, and (b) Array of oligonucleotides that capture molecules synthesized on the chip (http://www.combimatrix.com/tech_microarrays.htm).

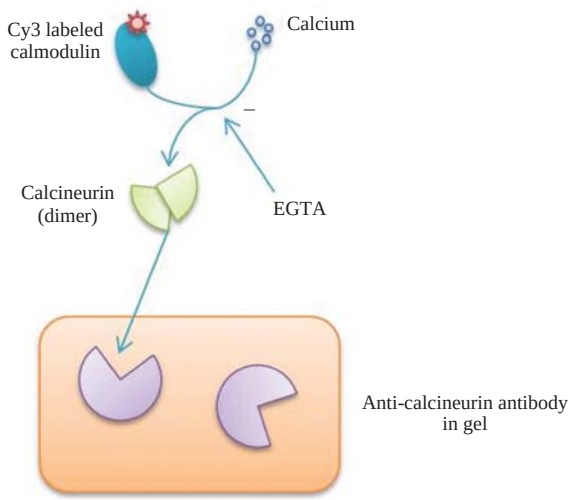

Figure 2. Multiple protein interactions mediated by small molecules in the hydrogel matrix: Anti-calcineurin antibody is directly immobilized in a hydrogel, then incubated with calcineurin, and a Cy3 labeled calmodulin that can be visualized by laser scanner binds to the calcineurin in the presence of Ca^{2+}. EGTA is used to increase the signal intensity.

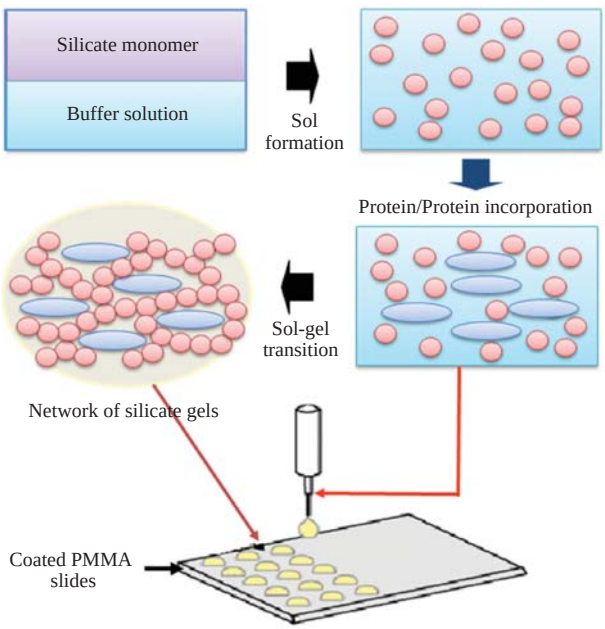

Figure 3. Three-dimensional nanoporous structured biochip: The hydrolyzed precursor is mixed with a buffered protein sample to obtain a three-dimensional silica network with immobilized biomolecules. The gel mixture is spotted onto PMMA-coated slides by a microarrayer.

or therapeutic targets.

Use of Three-dimensional Gels in Protein Chips

As the conformation of a protein is closely related to its functionality, the immobilization of the protein using the covalent binding of its functional site may lead to inactivation of the protein. Thus, an appropriate immobilization method or matrix is required. An optical transparent hydrogel, or sol-gel, such as polyethylene glycol (PEG), polypropylene glycol (PPG)-based polymeric microdoplets[9], or silicate monomers[10] are some preferred examples in the synthesis of three-dimensional microarrays. These three-dimensional gel structures contain pores and channels that are essential for retaining the bioactivity of a substrate protein encapsulated inside the gel. This unique structural characteristic provides a good chance to do an extensive study on the molecule interactions; for example, the interactions between antibody-antigen, protein-protein, protein-nucleic acid, protein-lipid, and protein-small molecule or enzyme-substrate[2].

By the invention of the isocyanate-functional hydrogel biochip[9] (Figure 2), a high resolution biochip that has as many as 1,000 individual reaction cells per square centimeter was produced. Assume at least one binding entity is immobilized within a microdroplet; by altering the binding entities in the cells, an efficient screening for binding interactions or activities can be obtained in this hydrogel biochip.

In the case of sol-gel, Kim *et al.*[11] developed a unique screening strategy to provide an optimized formation for embedded proteins or antibodies. The

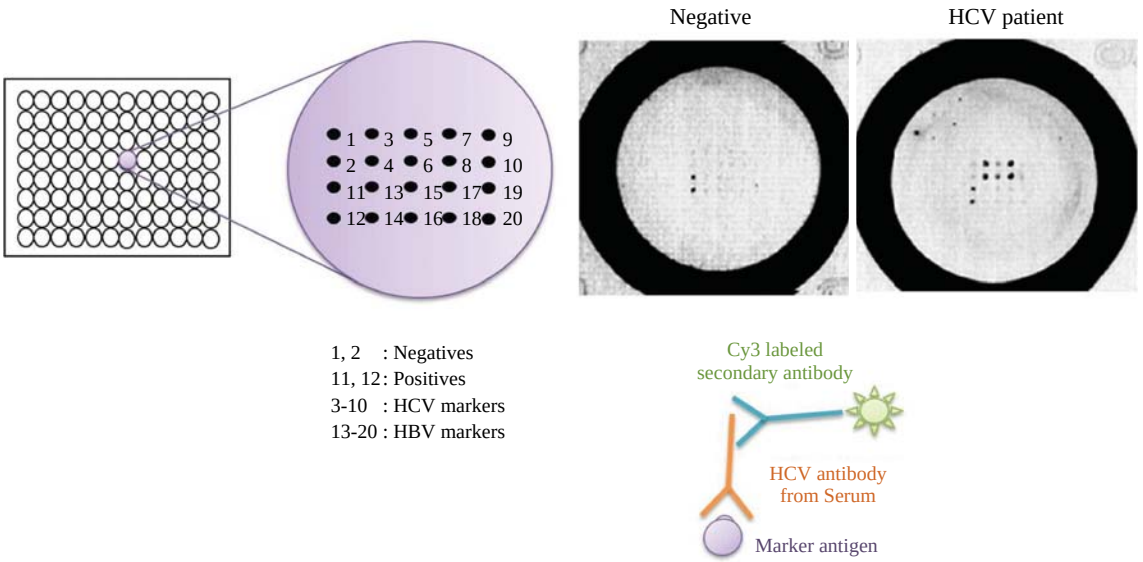

Figure 4. Sol-gel protein microarray for HCV detection: Diagnostic application with patient samples: the left side is a diagram of the diagnostic prototype within 8 mm 96-well plates: 1-2 Negative control, 11-12 positive control, 3-10 HCV markers (core, NS5, NS3, E1/E2), and 13-20 HBV markers; the right side are scanned images of two representative wells. Negative: a serum sample from a normal patient, Patient: a serum sample form an HCV-positive patient.

newly composed sol-gel-based protein chip had improved physical properties of sol-gel chips and showed femtogram-level sensitivity for the detection of protein-protein interaction.

Based on the optimized sol-gel formation (Figure 3), the marker antigens against HCV[12] were immobilized spots on a 96-well plate protein chip using a microarrayer (Figure 4). The fluorescent tagged antibodies interacting with their specific serum antibodies or proteins give out a fluorescent signal. This sol-gel chip resulted in a higher diagnostic accuracy (98.78%) than the conventional method currently used in HCV diagnosis, ELISA (81.71%). This is the first clinical application of the protein immobilized sol-gel chip, showing the possibility of a gel-derived three-dimensional microarray for biomedical research and diagnosis.

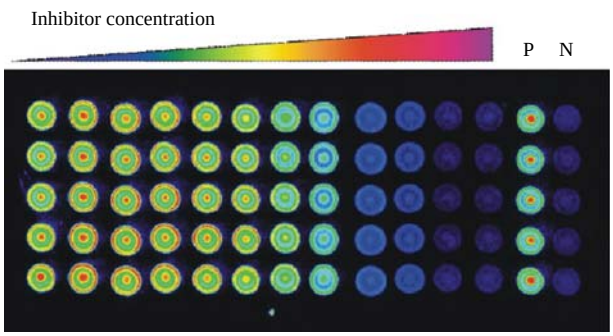

Figure 5. An inhibition assay performed on a kinase (PKA-kempitide) microarray. Inhibitor concentration increased from left to right, resulting in decreased fluorescence intensity due to inhibition of the phosphorylation reaction. N: BSA negative control, P: β-casein positive control (http://www.chemistry.mcmaster.ca/faculty/brennan/).

Use of Silica Porous Biochips for Drug Development

Conventional drug discovery involves a series of complex processes that are full of hidden obstacles, opportunities to fail preclinical trials, and even the risk of patients' lives. It has become critical for pharmaceutical companies to have cost-effective and safer ways to discover new drugs.

Using protein-entrapped nanoporous silica chips to screen various kinds of small molecules in order to make bioactive substrate libraries is rated as an economical and timesaving course of action in the pharmaceutical industry. This does not only reduce the volume of expensive biological reagents (sometimes proteins with low abundance) or compounds, but also gives an efficient high-throughput screening result[13].

Phosphorylation intricately concerned in a signaling pathway is one of the most common post-translational modifications of proteins[14]. Recent studies reported that kinases play an important role in the treatment of a wide range of human cancers[15], making them an attractive target for therapeutic approaches using a microarray.

Up to now, most reports have focused on arrays

containing substrates of target enzymes[16]. Only a few reports have been conducted on the use of microarrays of immobilized enzymes to screen inhibitors of critical enzymes such as phosphatases, serine hydrolases[17], and cysteine proteases[18], as well as proteins of the G protein-coupled receptor family (GPCRs)[19]. In a study from Rupcich *et al.*[20], a α-catalytic subunit of a cAMP -dependent protein kinase (PKA) and the peptide substrate kemptide were co-immobilized in a single-pin printed sol-gel derived microarray to monitor phosphorylation and inhibition. The multi-component kinase microarray could detect kinase inhibition even in a nanovolume (Figure 5). In another study done by Lee *et al.*[21], a series of membrane-associated P450 enzymes were encapsulated in sol-gel-based microarrays to analyze the drug metabolism by these enzymes.

Such type of high-throughput screen may offer the possibility to design personalized treatment regimens for patients, as well as for the identification of a safe pharmacology and advancement to clinical trials.

Conclusion

In additional to DNA, protein, and small molecules captured in a gel, another novel biochip is also being rapidly developed. Aptamers, single-stranded nucleotides selected using a SELEX[22] process, are suitable for applications in analytical[23], diagnostic, and therapeutic studies[24] based on their molecular recognition. In a recent study by Kim *et al.*, a sol-gel-derived RNA aptamer biosensor[25] was optimized against the detection of the hepatitis C virus[26].

The development of three-dimensional biochips gives the possibility of simultaneously screening with a wide range of purposes, ranging from environmental analyses on water pollutants, drug candidate screening in pharmacology, and diagnoses against specific pathogens in medical research. As the application of three-dimensional biochips expands further, the needs for profound researches on the optimization of the current material and the discovery of an epochal method will be expected.

Acknowledgements

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